Originals

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Spatial Genetic Structure in a Population of *Hemerocallis taeanensis* (Liliaceae)

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To examine neighbourhood size of *Hemerocallis taeanensis*, having a restricted distribution maintaining high level of genetic variation, spatial distribution of genotypes was analyzed using spatial autocorrelation of 27 alleles. Ninety-six individuals were mapped and sampled within a 10 × 20-m area in a population. Moran's *I* values were significantly different from the expected value in 31 (11.5 %) of 270 cases, and the overall correlogram was significant for eight (30 %) of 27 alleles. Mean correlogram indicates that the pattern across ten distance classes is somewhat circular or patch distribution is somewhat weak, but regular. These results indicate that pollen movement by bees between populations of *H. taeanensis* has been sufficient to retard intrapopulational genetic differentiation.

Hemerocallis taeanensis S.Kang & M.Chung (Liliaceae), a herbaceous perennial, is presently known only from grasslands under pine-oak coastal forests in Taean Gun, along the middle western Korean coast (Kang 1997). Individuals of the species have 2–5-flowers on an inflorescence. Flowers are orange-yellow, usually visited by bees (*Bombus diversus diversus* and *Apies mellifera*) and flies (Kang 1997) and start to open during the hours before sunrise and remain open until the afternoon. The flowering period is mid May to June in natural habitats. Like other *Hemerocallis* species, *H. taeanensis* has no specialized mechanism for seed (4 mm long and 3 mm wide) dispersal, and many seedlings found near maternal plants in natural populations (Kang and Chung pers. obs.). Chromosome number is 2n = 22 (Kang 1997).

Although *H. taeanensis* has a restricted distribution in Korea, populations of the species maintain high levels of genetic variation (mean expected heterozygosity, He = 0.231). This value is very comparable with those for more widespread congeners such as *H. hakuunensis* Nakai (He = 0.245) and *H. thunbergii* Baker (He = 0.265) (Kang 1997). As Holsinger and Gottlieb (1991) suggested that a narrowly distributed species with a large effective population size may resemble more widespread congeners in its amount of genetic variation, Kang (1997) suggested that *H. taeanensis* would have a relatively large neighborhood size. In this study, we analyze spatial genetic structure within a population of *H. taeanensis* using spatial autocorrelation analysis (Sokal and Oden 1978a) in an attempt to search for "neighborhood structure" or patches of genetically similar individuals of this species.

Materials and Methods

In June 1995, we mapped 96 flowering individuals of *Hemerocallis taeanensis* within a 10 × 20-m area located on Songhyun Ri, Sowon Myeon, Taean Gun, Prov. Ch'ungch'eongnam Do and collected their leaf samples. Leaf samples were wrapped in wet paper towels, placed in plastic bags and stored on ice to prevent protein denaturation prior to returning to a laboratory, where they were stored at 4°C until protein extraction. Leaf samples were cut finely, and crushed with a mortar and pestle. A phosphate-polyvinylpyrrolidone extraction buffer (Mitton et al. 1979) was added to the leaf samples to facilitate crushing and to aid enzyme stabilization. The cellular extract was absorbed onto 4×6 mm wicks cut from Whatman 3MM chromatography paper, which were then stored at −70°C until needed. Electrophoresis was performed using 11% starch gels. Fifteen putative loci were resolved from eight enzyme systems using three gel/electrode buffer combinations. Two Poulik buffer systems were used: a modification (Haufler 1985) of Soltis et al. (1983) system 6 was used to resolve leucine aminopeptidase (LAP), fluorescent esterase (FE) and a modification (electrode buffer of pH 8.6) of Haufler (1985) resolved diaphorase (DIA), alcohol dehydrogenase (ADH), and β-galactosidase (β-GAL). A morpholine citrate buffer system, a modification (Chung and Kang 1994) of that of Clayton and Tretiak (1972), was used to resolve malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), and 6-phosphogluconate dehydrogenase (6PGD). Stain recipes were taken from Soltis et al. (1983), except for the DIA and β-Gal, which were taken from Cheliak and Pitel (1984). The genetic basis of enzyme banding patterns was inferred from observed segregation patterns in light of typical subunit structure and subcellular compartmentalization (Weeden and Wendel 1989). Putative loci were designated sequentially, with the most anodally migrating isozyme designated '1', the next '2', and so on. Likewise, alleles were designated alphabetically starting from 'a' with the most anodally migrating allele. *Lap-2, Dia-2*, and *Pgd-2* loci were not included in this study due to faint or inconsistent staining. *Pgi-1* and *β-Gal* loci were monomorphic in all individuals. A locus was considered polymorphic in a population only if the most common allele occurred at a frequency of 0.95 or less (a 95% criterion).

For spatial autocorrelation analysis, the genetic data were coded so that allele frequency values of 0.0, 0.5, or 1.0 were assigned to individuals for allele of each locus (Sokal and Oden 1978a). Only one allele was considered at dialellic loci as the second allele would contribute identical information. Every possible pair of individuals was considered as a join and was assigned to one of the ten distance classes (Fig. 1), and the ranges of these were selected by equalizing sample sizes. Moran's I values (Sokal and Oden 1978a) were calculated for each of ten distance classes by interpopulational distance classes by

$$I = N\sum_{i}\sum_{j}(W_{ij}Z_iZ_j)\,(\sum_{i}\sum_{j}W_{ij}\sum Z_i^2)^{-1}$$

where, N is number of individuals, W_{ij} is the join on weighting matrix, where Wij is set as one if ith and jth individual are the distance class and zero otherwise, $Z_i = X_i - \overline{X}$, $Z_j = X_j - \overline{X}$, the variables X_i and X_j are the genotypic scores for ith and jth individuals, respectively, and $\overline{X}$ is the mean score for all individuals. Each I value was used to test significant deviations from the expected values, $E(I) = -1/(N-1)$ (Cliff and Ord 1981). A significant positive value of Moran's I indicates that the neighboring individuals in the distance class considered tend to have similar gene frequencies, whereas a significant negative value suggests that they tend to have different gene frequencies. Overall significance of individual correlograms was tested using Bonferroni's

criteria (Sakai and Oden 1983). All calculations and statistical analyses were performed using the SAAP program (ver. 4.3) written by D. Wartenberg.

Results

Twenty-seven alleles for *Hemerocallis taeanensis* were used for spatial autocorrelation analysis on the basis of 95% criterion for considering a polymorphic locus. The spatial autocorrelation coefficients, Moran's *I*, are presented in Table 1. The Moran's *I* values were significantly different from the expected value (E[*I*] = –0.011) in 31 (11.5%) of 270 cases, and the overall correlogram was significant for eight (30%) of 27 alleles (Table 1). From the distance classes 1 to 3 (0<2.8 m), seven significantly positive cases were observed, whereas four significantly negative cases were detected in the distance class 3. This indicates that a genetic similarity was shared among individuals within 2.8 m distance. On the other hand, *I* was significantly negative in nine cases beyond the distance class 5, indicating an overall genetic dissimilarity among individuals beyond the distance (Table 1). The average Moran's *I* values for each distance class were 0.03, 0.00, –0.02, –0.01, –0.02, –0.02, 0.01, –0.03, –0.02, and –0.02 (Table 1 and Fig. 1).

Discussion

The ratio of significant *I* values in *Hemerocallis taeanensis* was higher than the intended 5% type I error, suggesting that genetic structuring within the population existed for the species. Among 270 cases calculated for the ten distance classes in a population of *H. taeanensis*, Moran's *I* was significant for 31 (11.5%) cases. In addition, the number of significant overall correlograms was eight. It may be of interest to compare the present results with a previous study of another outcrossing liliaceous plant which has a similar life history and ecological traits with *H. taeanensis*. Maki and Masuda (1994) examined spatial genetic structure, using the same computer program as in this study, in two outcrossing populations (10×20-m and 2×20-m areas) of *Chionographis japonica* var. *japonica*. This species, occurring in understory of forests in western Japan, is a self-incompatible perennial. Bees visit flowers and no specialized mechanism of seed dispersal is known (Maki and Masuda 1994). Overall spatial genetic structure found in the species was much more distinct than that of *H. taeanensis*. Four Moran's *I* values were significantly positive of six cases in the shortest distance (0<1 m).

The distance at which mean Moran's *I* values first intercepts the E(*I*) value may represent the shortest side of an irregularly shaped patch size (Sokal 1979). The mean correlogram of a population of *H. taeanensis* indicates that the minimum patch size was approximately 5 m^2. However, the mean correlogram indicates that the pattern across ten distance classes is somewhat circular (Sokal and Oden 1978b) or patch distribution is somewhat weak, but regular. More recently, Kang (1997) revealed that indirect estimates of gene flow, as measured as *Nm* (number of migrants per generation) values, among four adjacent populations (about 30 km boundary) of *H. taeanensis* were high (4.70 calculated from mean Nei's [1973, 1977] G_{ST} and 6.35 calculated from Slatkin's [1985] private alleles). For neutral genes, *Nm* values below 1 indicate that genetic drift is the predominant factor affecting population substructure, whereas *Nm* values above 4 indicate that gene flow replaces genetic drift (Hartl and Clark 1989). As *H. taeanensis* has no specialized mechanism of seed dispersal, the considerably high levels of gene flow indicates that pollen flow may be in general sufficient to retard intrapopulational genetic differentiation in the species.

Table 1. Spatial autocorrelation coefficients (Moran's *I*) over ten distance classes for 29 alleles in a population of *Hemerocallis taeanensis*

Allele	1	2	3	4	5	6	7	8	9	10	P^a
Lap-1^c	0.09**	–0.02	–0.02	–0.08	–0.13**	–0.01	0.06*	–0.02	–0.00	0.03	0.030
Fe-1^d	–0.01	0.02	0.06*	–0.04	–0.01	–0.05	–0.03	–0.00	–0.04	–0.00	0.325
Fe-1^e	0.07*	0.02	0.05	–0.04	–0.04	–0.06	–0.03	–0.01	–0.04	–0.01	0.219
Fe-1^i	0.05	–0.00	–0.12**	0.06	0.02	0.05	0.03	–0.06	–0.06	–0.06	0.051
Fe-2^c	0.03	–0.05	–0.04	–0.03	–0.03	0.04	0.03	–0.01	0.01	–0.05	1.000
Fe-2^d	–0.00	–0.05	0.02	–0.07	–0.05	0.04	0.05	–0.01	–0.05	0.01	0.702
Fe-2^e	0.04	–0.03	–0.03	–0.03	–0.02	0.02	0.01	–0.02	–0.04	–0.01	1.000
Fe-2^f	–0.02	0.01	–0.00	0.01	–0.02	–0.01	–0.04	–0.07	0.07*	–0.02	0.240
Adh^e	0.02	–0.01	–0.06	–0.03	–0.03	0.02	0.02	–0.04	–0.04	0.04	1.000
Dia-1^a	0.00	–0.01	0.03	0.01	0.03	0.03	–0.03	–0.09*	–0.05	–0.03	0.198
Dia-1^c	0.03	0.01	–0.03	–0.02	0.03	–0.08	0.09*	–0.01	0.01	–0.14**	0.002
Dia-1^e	0.05	0.02	–0.04	–0.07	0.02	0.00	0.09**	–0.01	0.03	–0.19**	0.000
Dia-1^g	–0.01	0.05	–0.09*	–0.08	0.05	0.03	0.03	–0.01	–0.07	0.00	0.296
Mdh-1^d	–0.01	0.03	–0.05	–0.05	0.05	–0.04	0.01	0.01	0.01	–0.07	0.575
Mdh-2^d	–0.02	0.05	–0.05	–0.06	0.01	–0.04	0.03	–0.03	–0.02	0.02	0.624
Pgd-1^c	0.01	0.03	–0.01	0.03	–0.00	–0.02	0.01	–0.04	–0.06	–0.05	1.000
Pgd-1^g	–0.01	–0.02	–0.14*	0.03	0.07*	0.02	–0.04	–0.05	–0.00	0.03	0.016
Pgd-1^f	–0.03	–0.02	–0.11**	0.11**	0.06	–0.04	0.02	–0.05	–0.09*	0.03	0.020
Pgi-2^b	–0.04	0.04	0.05	–0.05	–0.04	–0.04	0.02	–0.05	–0.01	0.02	0.826
Pgi-2^g	–0.02	–0.02	0.07*	0.06*	–0.08	–0.05	0.03	–0.07	–0.07	0.04	0.353
Pgi-2^i	0.06*	0.02	–0.03	0.03	–0.07	–0.07	–0.02	–0.06	–0.07	0.10**	0.010
Pgi-2^k	0.13**	0.01	–0.05	–0.02	–0.03	0.01	–0.03	–0.05	0.00	–0.10*	0.004
Pgi-3^a	0.04	0.04	–0.05	–0.04	–0.07	0.00	–0.10*	0.00	0.01	0.04	0.226
Pgi-3^c	0.15**	–0.05	–0.01	0.07*	–0.06	–0.03	0.07*	–0.01	–0.07	–0.17**	0.000
Pgi-3^e	0.04	–0.02	–0.02	0.09*	–0.04	–0.11*	–0.02	0.06	–0.03	–0.04	0.136
Pgi-3^g	0.05	0.03	–0.00	–0.06	–0.07	–0.05	–0.00	–0.05	0.03	0.02	0.623
Pgi-3^i	–0.00	0.01	0.04	–0.01	–0.04	–0.04	–0.06	0.02	–0.01	–0.02	0.725
Average	0.03	0.00	–0.02	–0.01	–0.02	–0.02	0.01	–0.03	–0.02	–0.02	

[a]Overall correlogram significance (Bonferroni approximation). * = $P < 0.05$; ** = $P < 0.01$.

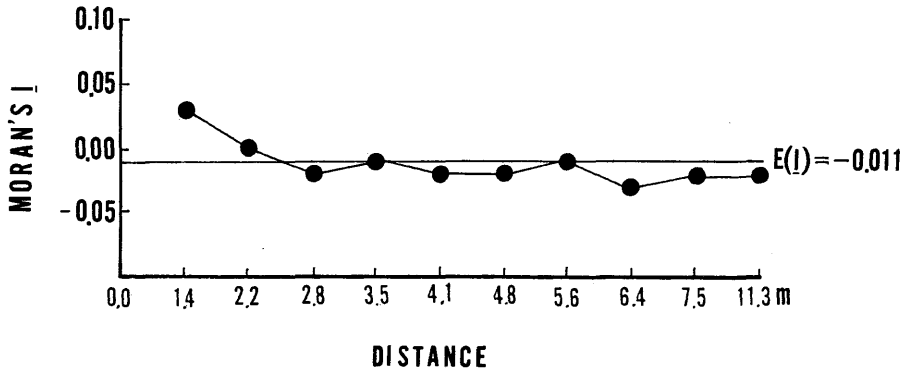

Fig. 1. Mean correlogram for a population of *Hemerocallis taeanensis.*

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朴 祺培[a], 姜 順淑[b], 鄭 明基[b]: ***Hemerocallis taeanensis*（ユリ科）集団の空間的遺伝構造**

忠清南道Songhyun里における*Hemerocallis taeanensis*集団（10×20 m）の96個体の27対立形質について，個体間の距離による交流度を空間的自己相関係数（Moran's *I*）を用いて検定した．係数の値は検定した270例の11.5%について期待値より有意差があり，全体としての相関は27対立遺伝子の30%について有意であった．この結果は花粉の移動が十分行われていて，集団内での遺伝的分化を抑えていることを推定させる．

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第73巻3号 正誤 Errata of Vol. 73 No. 3

頁(Page)	行(Line)	誤(For)	正(Read)
143	↓ 17	and all *Elliottia*	and *Elliottia*
143	↓ 22	the subgenus Tsutsusi	subgenus Tsutsusi
153	↑ 6	高柳謙二	高柳謙治